brief communication

Measuring local surface charge densities in electrolyte solutions with a scanning force microscope

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ABSTRACT To show that local surface charge densities can be measured with a scanning force microscope purple membranes adsorbed to alumina were imaged in electrolyte solutions. Force versus distance curves were measured on purple membranes and on the bare alumina with standard silicon nitride tips. By comparing the electrostatic force measured on both substances, the surface charge density of purple membranes could be calculated from the known charge density of alumina. The charge density of purple membranes was estimated to be -0.05 C/m^2.

INTRODUCTION

The scanning force microscope (SFM), invented by Binnig et al., 1986, has become an important tool for imaging surfaces (Wickramasinghe, 1989). In the SFM, a sharp tip at the end of a cantilever is scanned over a surface. While scanning, surface features deflect the tip and thus the cantilever. By measuring the deflection of the cantilever, a topographic image of the surface can be obtained. Beside the ability of SFMs to image surface topographies down to atomic resolution, it is possible to obtain information about local surface properties like magnetization (Martin and Wickramasinghe, 1987; Sáenz et al., 1987; Mamin et al., 1988), conductance (Morita et al., 1989), or the elasticity of the sample (Burnham and Colton, 1989; Maivald et al., 1992). Stern et al. (1988) and Terris et al. (1989) were able to create and image electric charges with a nickel tip on insulators in air.

In this paper I describe measurements of the surface charge density of purple membranes in electrolyte solutions. Purple membranes are membrane fragments of ~0.5-μm diameter and 5-nm thickness (Oesterhelt and Tittor, 1989). They are isolated from the plasma membrane of *Halobacterium halobium* and are stable up to pH 12 (Brouillette et al., 1987). To measure the charge density of purple membranes they were deposited onto alumina. Alumina was chosen because its surface charge is known and changes drastically with pH (Ahmed, 1969; Schwarz et al., 1984; Sprycha, 1989). By choosing a certain pH the charge density of alumina could be selected.

After imaging purple membranes adsorbed to alumina, force versus distance curves were measured on bare alumina and on purple membranes. Beside the van der Waals (vdW) attraction, an electrostatic force was observed (see also Ducker et al., 1991; Butt, 1991*a*). The electrostatic force can be approximated by (Butt, 1991*b*):

$$F_e = \frac{4\pi}{\epsilon_0 \epsilon \kappa^2} \cdot [g_1(\sigma_T^2 + \sigma_S^2) \cdot e^{-2D\kappa} + g_2 \cdot \sigma_T \sigma_S \cdot e^{-D\kappa}]. \quad (1)$$

σ_T and σ_S are the charge densities of tip and sample surface, ϵ_0 and ϵ represent the vacuum permittivity and the dielectric constant of water. D is the distance between tip and sample. g_1 and g_2 are geometry factors which only depend on the tip shape. κ is the inverse of the Debye length. For a monovalent salt of concentration C (in mol/l) and at 22°C it is given by

$$\kappa^{-1} = \frac{0.304}{\sqrt{C}} \text{ nm}. \quad (2)$$

By comparing the electrostatic force obtained on purple membranes with those obtained on alumina, the charge density of purple membranes could be determined. This direct comparison ensured that the tip shape, the charge density of the tip, and the salt concentration did not influence the results. All experiments were done with standard silicon nitride tips in 2 mM NaCl at various pH values. Due to 1 mM buffer, the ion strength was between 2 and 3 mM.

MATERIALS AND METHODS

A commercial SFM (Digital Instruments, Santa Barbara, CA 93017) including control electronic and software was used to measure force versus distance curves. A force versus distance curve displays the deflection of the cantilever, on which the tip is mounted, as a function of the vertical position of the *xyz* translator, on which the sample is mounted. The deflection of the cantilever is proportional to the force. The *xyz* translator moves the sample up and down with constant speed. This movement is induced by applying a voltage to the piezoelectric element, which is responsible for the *z* position. The total time for a complete up and down cycle was ~1 s. To record force versus distance curves, the deflection signal of the cantilever and the *z* voltage of the *xyz* translator were fed into two channels of a digital oscilloscope. Averaged signals were later transferred to a computer and the approaching part of force versus distance curves was further analysed (for details see Butt, 1991*a*).

The curves were corrected for the additional distance due to cantilever deflection. Therefore, I added to all distance values the corresponding cantilever deflection divided by the sensitivity. The sensitivity was obtained in the contact regime of the force versus distance curve.

Flat pieces of alumina ($\gamma - Al_2O_3$, Aldrich Chemical Co., Steinheim, Germany) were mechanically attached to Teflon discs, which were glued to a steel disc to mount the sample on the *xyz* translator. The silicon nitride cantilevers were 200 μm long and had a spring constant of 0.032 N/m or 0.064 N/m (Digital Instruments Inc.).

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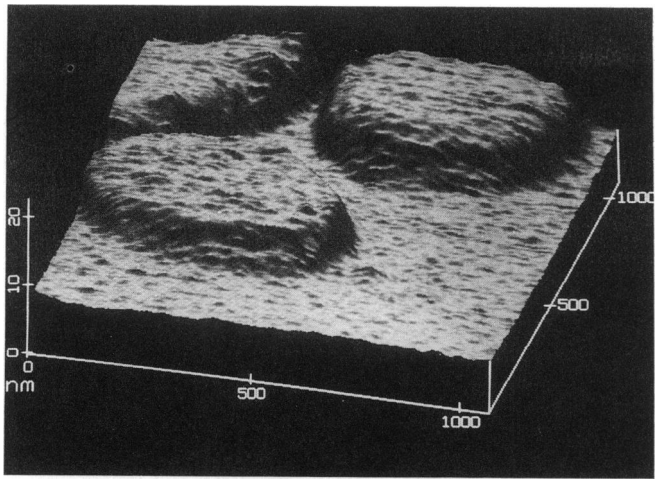

FIGURE 1 Purple membranes adsorbed to alumina and imaged with an SFM after measuring the force versus distance in 2 mM NaCl, 1 mM MES at pH 6.1.

Purple membranes were prepared from *Halobacterium halobium* strain S9 according to Oesterhelt and Stoeckenius, 1974. 50 μl of purple membrane solution at a concentration of 40 μg/ml (in 40 mM NaCl, 1 mM $CaCl_2$, pH 6) was applied by pipette onto clean alumina. After 10 min, the rest of the volume was filled with pure buffer. Once adsorbed, purple membranes stayed on the surface and the buffer could be exchanged in the flow-through imaging cell. Measurements were done in solutions containing only monovalent salts. To ensure that the free calcium concentration was negligible, the cell was rinsed with ~10 ml of the new buffer, which corresponded to about 200 times the cell volume.

Chemicals were grade p.a. and bought from Merck (Darmstadt, Germany), Roth GmbH (Karlsruhe, Germany), or Sigma Chemical Co. (St. Louis, MO). The water was deionized (resistivity: 18 MΩ cm). The following buffers were used: Citric acid (pH 3.2), 2-[*N*-Morpholino]ethanesulfonic acid (MES, pH 6.1), 3-(*N*-Morpholino)propanesulfonic acid (MOPS, pH 7.3), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES, pH 7.7), Tris-(hydroxymethyl)-aminomethan (TRIS, pH 8.1), 2[*N*-Cyclohexylamino]ethane-sulfonic acid (CHES, pH 9.3), and sodium carbonate (pH 10.3).

RESULTS AND DISCUSSION

An image of purple membranes adsorbed to alumina is shown in Fig. 1. Once the set up is thermally stable, samples could be scanned for hours without destroying anything. Alumina often displayed surfaces with a roughness below 2 nm (measured over 1 × 1 μm). This allowed to make force versus distance measurements with an accuracy of 2 nm. Many biomolecules are negatively charged. Hence, alumina, with its positive surface charge at neutral pH, might be a suitable substrate to fix biological samples.

A typical series of force versus distance curves obtained on purple membranes (*continuous lines*) and on the bare alumina (*dotted*) is shown in Fig. 2. At pH 10.3 it can be seen that the tip before touching the surface was repelled. At pH 9.3 and pH 8.1 a repulsion was observed at large distances. Closer to the sample, the tip was attracted and jumped onto the sample. When the pH was further lowered purely attractive forces were measured. On purple membranes the repulsion also decreased, but down to pH 6.1 no attraction was observed. At pH 3.2 sometimes a small attractive component was present but usually curves looked like the one shown in Fig. 2.

In the attractive region, force curves might not display the true force versus distance relation. Usually the tip jumped onto the sample at a certain distance. That smooth curves are shown in Fig. 2 even in the attractive region is due to averaging and the correction for cantlever deflection.

Out of six experiments in three cases, the whole pH variation could be done. In the other experiments obvious problems occurred, which were probably caused by contaminats. That contamination often disturbed measurements was concluded from experiments on bare alumina without purple membranes. In such experiments, problems could often be solved by rinsing with water, ethanol, and hexane.

The observed variation of the force with changing pH can be explained with the electrostatic and the vdW force. The vdW attraction is independent of pH, but the electrostatic force changes as the surface charge densities of the silicon nitride tip, alumina, and purple membranes change. Silicon nitride is probably oxidized at the surface and bears a negative charge density which increases with increasing pH (Abendroth, 1970; Schwarz et al., 1984; Maier et al., 1987; Butt, 1991*a*). Alumina is positively charged at low and neutral pH. Around pH 8 it is neutral, above pH 8 it bears a negative surface charge (Ahmed, 1969; Schwarz et al., 1984; Sprycha, 1989). Consequently, above pH 8, a repulsive electrostatic force is expected on alumina, below pH 8 the tip should be attracted. This was observed. The attractive component at close distances at pH 9.3 and at pH 8.1 can be explained with the vdW attraction.

Purple membranes are negatively charged above pH 3 (Carmeli et al., 1980; Packer et al., 1984). Hence, the electrostatic force should be repulsive, which was observed. At pH 3.1 the influence of the vdW force can be seen. Usually the vdW attraction just compensated the electrostatic repulsion. On purple membranes the vdW attraction was weaker than the attractive component observed on alumina.

The electrostatic force between tip and sample was always dominated by the second term in Eq. 1. This was deduced from the distance dependence. When fitting force versus distance curves with the exponential function

$$F(D) = F_0 \cdot e^{-D/\lambda}, \tag{3}$$

(see Fig. 2 at pH 10.3 and pH 9.3), decay constants λ of 7 ± 2 nm were obtained. This agrees with the Debye length of 6.8 nm calculated with Eq. 2. As the first term in Eq. 1 contains a decay length of half the Debye length,

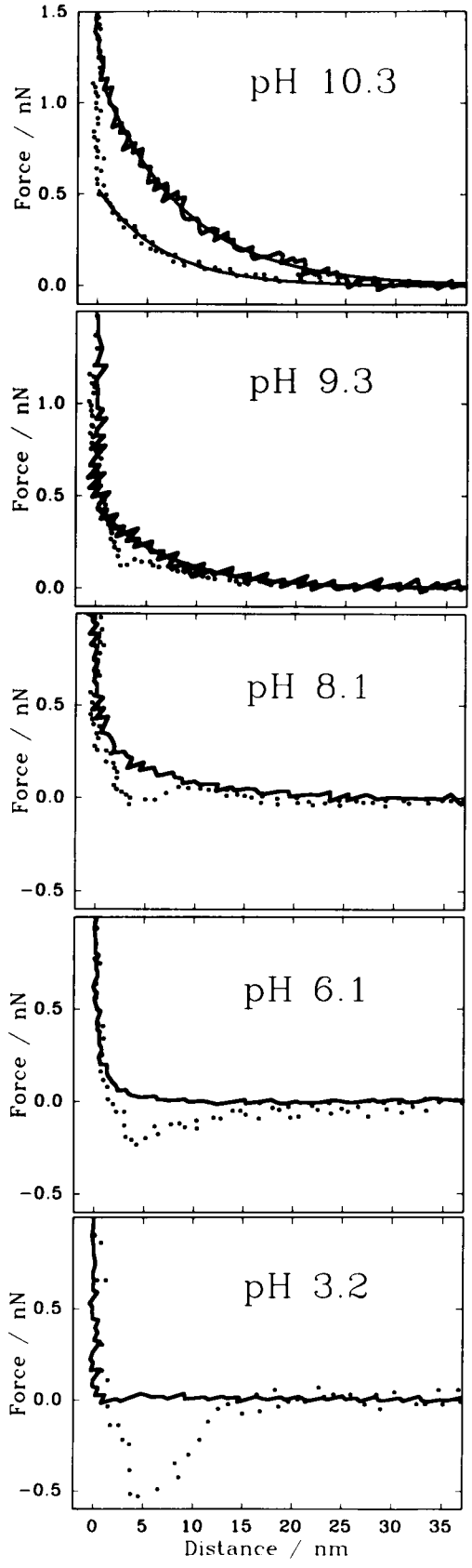

FIGURE 2 Force between tip and sample versus distance measured on alumina (*dotted*) or on purple membrane (*continuous line*) in 2 mM NaCl plus 1 mM sodium carbonate (pH 10.3), CHES (pH 9.3), TRIS (pH 8.1), MES (pH 6.1), or citric acid (pH 3.2). Positive forces are repulsive, negative forces are attractive. The thin, continuous lines shown at pH 10.3 and at pH 9.3 for purple membranes were obtained by fitting with the exponential function 3. Standard silicon nitride tips were used. To account for the additional distance due to cantilever deflection the curves were corrected. This correction caused a noise in the horizontal direction. The signals were four times averaged.

its influence is probably negligible. Like in previous experiments (Butt, 1991*a*) the decay length increased when lowering the salt concentration and it decreased at high salt concentration.

When only the second term in Eq. 1 is considered, the ratio of the force amplitudes obtained on alumina F_{al} and on purple membrane F_{pm} is related to the charge densities on both materials by

$$\frac{\sigma_{pm}}{\sigma_{al}} = \frac{F_{pm}}{F_{al}}, \tag{4}$$

σ_{al} is known (Ahmed, 1969; Schwarz et al., 1984; Sprycha, 1989). With F_{pm} and F_{al} obtained from fitting the corresponding force versus distance curves, σ_{pm} could be calculated. Charge densities of alumina and charge densities of purple membranes calculated with Eq. 4 are given in Table 1.

For pH 8.1 and 6.1 the charge density of purple membranes could not be determined in direct comparison with alumina. Force versus distance curves on alumina were dominated by attractive forces and the electrostatic component could not be separated from the vdW attraction. In this case I assumed that the surface charge density of silicon nitride at pH 8.1 and 6.1 is the same as at pH 9.3. Then σ_{pm} could again be calculated with σ_{al} and F_{al} from the measurements at pH 9.3. However, the charge density of the tip decreases with decreasing pH. Hence, the values calculated at pH 8.1 and pH 6.1 are a lower limit for the amount of the charge density of purple membrane.

The surface charge density of purple membrane is still under debate. As the lipid composition and the amino acid sequence of the only protein, bacteriorhodopsin, are known (Khorana et al., 1979; Ovchinnikov et al., 1979; Kates et al., 1982; Renthal and Cha, 1984), one can calculate the charge density. At neutral pH purple membranes are expected to bear a charge density of ~ -0.22 C/m^2 intracellular and -0.08 C/m^2 extracellular. At pH 10.3 the charge density should have increased to -0.31 C/m^2 and -0.18 C/m^2, respectively. However, cations bind to purple membranes thus reducing the charge density. Results of measurements of the total charge density fall into two categories (Renthal, 1989). Kuschmitz and Hess (1981) and Renthal and Cha (1984) measured a high charge density of ~ -0.16 C/m^2. Other authors report charge densities around -0.007 C/m^2 (Ehrenberg and Meiri, 1983; Ehrenberg and Berezin, 1984; Packer et al., 1984; Kamo et al., 1987; Taneva et al., 1987). Correspondingly, the difference in charge densities between

TABLE 1 **Surface charge densities of alumina σ_{al} and purple membranes σ_{pm} at various pH values**

pH	6.1	8.1	9.3	10.3
σ_{al} in C/m^2	+0.01	−0.01	−0.04	−0.08
σ_{pm} in C/m^2	≥ −0.03	≥ −0.05	−0.06	−0.18

Charge densities for alumina are a mean of values obtained from Ahmed (1969), Schwarz et al. (1984), and Sprycha (1989). Charge densities of purple membranes in 2 mM NaCl were calculated with equation 4. At pH 8.1 and pH 6.1 the total charge densities of purple membranes given are a lower limit; the amount of σ_{pm} might be higher.

the intra- and the extracellular side was measured to be 0.069 C/m^2 (Keszthelyi, 1980) or around 0.006 C/m^2 (Barabás et al., 1983; Kimura et al., 1984; Papp et al., 1986; Taneva et al., 1987).

Results of my charge density measurements of purple membranes varied by 50%. If this large error was due to the fact that different sides of purple membranes were imaged or if it was the random error of the measurement can not yet be decided. Another uncertainty is the charge density of alumina taken from the literature. Ahmed (1969) Schwarz et al. (1984), and Sprycha (1989) measured the charge density of alumina by potentiometric titration in 1 mM KNO_3, 1 mM $NaNO_3$, and 10 mM NaCl, respectively. Their results varied by a factor two.

After the experiments one of the tips used was imaged with the SFM. From these images I estimated the interacting area on the sample and on the tip which contributed to the electrostatic force, assuming that both tips had the same shape. I further assumed that only those parts of the tip interact with the sample which are less than the Debye length of 6.8 nm away from the surface. This cross-sectional area of the tip at a height of 6.8 nm was ~1,300 nm^2, corresponding to a diameter of 40 nm. A similar value was obtained when the steepness of the edge of purple membranes was used to estimate the tip shape. Hence, 40 nm is about the lateral resolution of this charge measurement with an SFM. An area of 1,300 nm^2 bears 300 unit charges at pH 9.3 on alumina. The charge resolution is certainly lower than 300 unit charges (or 0.06 C/m^2) and limited by the vdW attraction. Once the attraction dominates the total force, the electrostatic component can no longer be determined.

A possible source of a misinterpretation is the influence of the vdW force. To exclude such an influence only distances larger than 3 nm from the jump-in point or from contact were considered in the fit with an exponential function. The vdW force is proportional to $1/D^{1\cdots2}$ (Israelachvili, 1985). All curves could be fitted with a single exponential and no $1/D^{1\cdots2}$ function was necessary. This indicates that a possible influence of the vdW force is negligible.

Eq. 1, on which the interpretation is based, is strictly only valid for surface potentials below 25 mV, for $D \gg \kappa^{-1}$ and for fixed, constant surface charges. These assumptions were not fulfilled at high pH. Fortunately, in these comparative measurements only the proportionality between charge density and force amplitude must be valid. Conditions for this proportionality are less severe than conditions for the whole equation 1 (Ring, 1985; Butt, 1991*b*). Still, a better understanding of the electrostatic force is obligatory before precise, quantitative experiments can be done.

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